Risdiplam: Editing on the RNA level – a dream or a nightmare? – Spinal Muscular Atrophy as example

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Spinal Muscular Atrophy (SMA)
A terminal disease with huge unmet medical need
A three party collaboration

A joint effort between three parties to develop a life changing treatment for children with SMA with urgency!
A protein called SMN, “Survival of Motor Neuron”
Spinal motor neurons are dependent on SMN protein

- Reduced level of SMN protein is responsible of SMA disease
- Essential in all species
- Adequate SMN levels are critical for normal development and functioning
- Reduced SMN levels result in loss of motor neurons in the spinal cord & progressive muscle atrophy
SMA disease mechanism: Every species has the SMN1 gene but the SMN2 “rescue” gene is human-specific

**Healthy subjects**

![Diagram of DNA, mRNA, and Protein showing SMN1 and SMN2 genes and their expression levels.](image)

- **DNA:** Fully functional
- **mRNA:** 70-90% stable, 10-30% degraded
- **Protein:** 95% fully functional, 5% rapidly degraded
SMA disease mechanism

SMA patients have in average 2-4 copies of SMN2 gene, this influences SMN protein levels and disease severity.
SM A disease mechanism

**Hypothesis**

<table>
<thead>
<tr>
<th>DNA</th>
<th>SMN1</th>
<th>SMN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;95%</td>
<td>stable</td>
<td>stable</td>
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</table>

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Ex.7</th>
<th>SMN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>unstable</td>
<td>Up to 95%</td>
<td>rapidly degraded</td>
</tr>
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<table>
<thead>
<tr>
<th>Protein</th>
<th>SMN1</th>
<th>SMN2</th>
</tr>
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<tbody>
<tr>
<td>stable</td>
<td>stable</td>
<td>SMNΔEx7</td>
</tr>
</tbody>
</table>

Drug
SMN2 gene copy: it is human specific, there is no animal correlate!!

Is what we see in animals translatable?
In terms of alternative splicing, probably yes, as this is highly conserved!
Animal findings: on MoA (splice modification) but off-target

- Justification of animal species and safety monitoring in patients based on secondary (off) splice targets
- Comparison of affected splice sites (with exon inclusion) across the genome
- Comparison of animal cells with patients cells to guide translatability understanding
Modifiers of SMN2 splicing as investigated by Roche

Science: August 2014, Vol. 345, Issue 6197

SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy

Nature Comm. 2017, Vol. 8, 1476

Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers

Small molecule SMN2 splice modifiers increase SMN2 protein and slow/halt disease in transgenic mouse model

Characterization of the binding sites of small molecule SMN2 splice modifiers, differences in specificity (splicing events and transcript changes) between molecules
Risdiplam (RG7916): oral, systemically distributed, investigational molecule in development for SMA

Risdiplam¹,²

- An investigational, selective SMN2 splicing modifier designed to bind uniquely with specificity to SMN2 pre-mRNA
- Promotes the inclusion of exon 7 in SMN2 mRNA and the production of full-length SMN2 mRNA and functional SMN protein
- Orally administered with a systemic distribution
- Penetrates the blood–brain barrier
- Developed in collaboration with:

mRNA, messenger ribonucleic acid; SMA, spinal muscular atrophy; SMN, survival of motor neuron.
Risdiplam increases lead to increased SMN2 mRNA and SMN protein levels

**SMN2 mRNA change in SMA Type 1 fibroblasts**

**SMN protein change in SMA Type 1 fibroblasts**

**SMN protein change in SMA Type 1 motor neurons**

Risdiplam increases lifespan and body weight in Δ7 mouse model of SMA

Body weight is increased in SMNΔ7 mice with risdiplam treatment

In vivo efficacy of risdiplam: survival benefit in SMNΔ7 mice into adulthood

HET, heterozygote/control littermate; IP, intraperitoneal injection; PO, oral gavage; SEM, standard error of the mean; SMA, spinal muscular atrophy; SMN, survival of motor neuron; SMNΔ7, truncated SMN protein.

Transcriptome Diversification by alternative Splicing

Central Dogma of Molecular Biology

Alternative Splicing

Li HD et al. (2014) The emerging era of genomic data integration for analyzing splice isoform function. Trends in Genetics

Buljan M et al. (2012) Tissue-Specific Splicing of Disordered Segments that Embed Binding Motifs Rewires Protein Interaction Networks. Cell
Specifcity of splice modification: a key safety optimization goal

Sivaramakrishnan et al., Nature Comm. 2017, Vol. 8, 1476

Two SMN2 small molecule splice modifiers:

SMN-C3 and NVS-SM1

- Overlapping pharmacophore
- Profound differences in splicing events
- Profound differences in transcript changes
- Profound differences in specificity
- Profound differences in safety?
Risdiplam has high specificity for SMN2 pre-mRNA

- Risdiplam binds two sites in SMN2 pre-mRNA
  - 5’ splice site (5’ ss) of intron 7
  - Exonic splicing enhancer 2 (ESE2) in exon 7
- Binding to the 5’ ss of intron 7 improves recognition by U1 snRNP
- U1 snRNP promotes inclusion of exon 7
- The unique specificity of binding two sites increases levels of full-length SMN mRNA and protein while reducing the impact on splicing of other pre-mRNA

ESE, exonic splicing enhancer; snRNP, small nuclear RNA; SMA, spinal muscular atrophy; SMN, survival of motor neuron; ss, splice site.
Discovery of FoxM1 as an additional gene spliced
A master regulator of cell cycle

- High expression and activity during S/G2/M phase
- No or low expression in non-dividing cells
- Critically required for execution of cell division
- FoxM1 blockade/knock-down induces mitotic arrest, micro-nucleation and apoptosis in proliferating cells

- Secondary pharmacology translates from human in-vitro (SMA patient derived fibroblast) to cynomolgus monkey in vivo (spleen)
SMN vs FoxM1 affinity: A striking correlation
Potential improvement vs RG7800

Defining a new Target Compound Profile (TCP)

In-vivo mouse Δ7; Ex-vivo hum. WB

Similar or superior to RG7800

Secondary splice target toxicities:
- Micronucleation in bone marrow
- GI tract
- Testes (germ cell arrest)

Higher window vs FoxM1 related toxicities

- Phototoxicity
- hERG (slight QTc prolong.)
- Phospholipidosis

No other safety concerns

- Vss= 15 L/kg (exp hum.);
- T₁/₂= 120h

No DM PK concerns
Prevention of Foxic non-brain penetrant major metabolite

Three possibilities

- Extremely broad range of basic amines are tolerated
- Needs to narrow down the possibilities
Optimizing Pharmacokinetics: How to reduce the Vss and prevent P-gp

**Human P-gp ER vs. LogD**

- Optimal range of LogD: > 1.5 (absence of human ER) < 3.0 (low Cl, good fu)

**Volume of distribution Vss in rat vs cpKa**

- Optimal range of cpKa: > 6.7 (SMN potency) < 8.5 for Vss

Novel compounds to be designed with a pKa between 6.7-8.5 and LogD between 1.5-3.0
Discovery of Risdiplam

**Lower basicity:**
- Reduced Vss
- No hERG
- No phospholipidosis

No "foxic" metabolite

No phototoxicity risk

### Table: Properties of RG7800 and Risdiplam

<table>
<thead>
<tr>
<th>Cpd</th>
<th>ΔΔG</th>
<th>mpKa</th>
<th>Vss (L/kg; cyno)</th>
<th>hERG IC20 μM</th>
<th>Phototoxicity IC50 μM</th>
<th>In vivo Efficacy (PDΔ7) fAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG7800</td>
<td>-4.5</td>
<td>10.9</td>
<td>20</td>
<td>0.3</td>
<td>0.3</td>
<td>0.112</td>
</tr>
<tr>
<td>risdiplam</td>
<td>-3.3</td>
<td>6.8</td>
<td>2.0</td>
<td>&gt; 10</td>
<td>&gt; 9</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Upon oral administration, risdiplam distributed to key tissues and organs that could be affected in SMA.

- Total plasma concentration similar to those in brain and muscle.
- CSF drug level were within the range of free compound in plasma.
Risdiplam Generates Similar SMN Protein Level Increases in the Periphery and in the CNS in Mouse Models of SMA

- Once daily treatment of two SMA mouse models* resulted in:
  - a similar dose-dependent increase in SMN protein levels in muscle (periphery) and brain (CNS)
  - a similar correlation between the increase in SMN protein in blood (periphery) and in brain† or spinal cord‡ (both CNS)

The increase in SMN protein in the periphery (e.g. blood of patients) can be used as a surrogate, or biomarker, as it reflects increases of SMN protein in the CNS

*Δ7 SMA mice and C/C-allele mice; SMN protein level in Δ7 SMA mice and C/C-allele mice treated with either
†RG7916 or ‡RG7800 (data not shown) and calculated as a percentage of SMN protein level in respective heterozygous mice.
Roche, a recognized leader in the field


Other manuscripts under preparation
Discovery of risdiplam: A success through an amazing collaboration between top scientists from PTC Therapeutics, SMA Foundation and Roche

Linda Burdette
Aude Clement
Christian Czech
Christine Freilinger
Irene Gerlach
Olaf Grassmann
Ricardo Hermosilla
Philippe Jablonski
Omar Khwaja
Heidi Kletzl
Stephane Nave
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Ying Qian
Sara Sunshine
Virginia Le Verche

& many additional scientists at Roche, PTC, and academic partners
Doing now what patients need next